Iranian Journal of Veterinary Science and Technology

Received: 2026- Mar- 07
Accepted after revision: 2026- Jun-02
Published online: 2026-Jun-03

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2026.97643.1639

Effects of intracerebroventricular injection of nisin on feeding behavior, body temperature, and serum parameters in LPS-inflamed broiler chickens

Vahid Hamedianasl, Farshid Hamidi, Hamid Reza Kazerani

Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

ABSTRACT

Lipopolysaccharide (LPS) leads to impaired production performance, reduced feed intake, altered body temperature, and deleterious changes in some serum and disease parameters in broiler chickens. Bacteriocins such as nisin are considered promising therapeutic agents for the prevention, control, and treatment of poultry diseases. This study involved an experiment using 30 male broiler chickens to evaluate the central effects of nisin on feed and water consumption, body temperature, and serum factors under stress. In this experiment, nisin (1 µg) and LPS (25 ng) were injected intraventricularly, and combined treatments of nisin and LPS were also considered. Feed intake, water consumption, and body temperature were measured in all groups. At the end of the experiment, blood was collected from the chickens for evaluation of serum factors. ICV administration of nisin administration significantly increased feed intake, whereas LPS administration significantly decreased it. In combination treatments, nisin was able to partially reduce the adverse effects of LPS on feed intake. Both nisin and LPS reduced water intake in broilers, and combined treatment caused a greater reduction in water intake than either injection alone. LPS initially reduced body temperature before hyperthermia induction, whereas nisin alone had no effect on thermoregulation, but in the combination groups, it somewhat reduced LPS-induced temperature disturbances. LPS also caused a significant decrease in albumin and a significant increase in AST and ALT, whereas nisin reduced AST and ALT but had no effect on albumin. Results shows that nisin can be used as a useful and safe additive in poultry feed.

Keywords

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

Number of Figures: 3
Number of Tables: 2
Number of References:: 49
Number of Pages: 11

Abbreviations

ICV: intracerebroventricular
IV: intravenous
LPS: lipopolysaccharide
ARC: arcuate nucleus

Corresponding author: *Farshid Hamidi*
Email: *Farshidhamidi@um.ac.ir*
Tel:+98(91)5511-2792

Introduction

Feed consumption in poultry is regulated by a multifaceted network of central and peripheral control systems driven by hormonal, metabolic, neural, and environmental factors [1]. Among the central regulatory structures, the hypothalamus is the endocrine host of feeding behavior and energy homeostasis [2]. Various hypothalamic regions, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventromedial hypothalamus (VMH) and dorsomedial nucleus (DMN) are known contributors of this regulatory mechanism [3]. These nuclei respond to circulating hormones and metabolites and subsequently regulate feeding behavior and body temperature [4].

In intensive poultry-rearing systems, broilers are continuously expose to environmental stressors and pathogenic microorganisms, that can suppress immune system activities and reduce productive performance [5]. Lipopolysaccharide (LPS) is an important component in the cell wall structure of Gram-negative [6]. Following entry of LPS into the broiler chicken's body, the animal undergoes an extensive acute phase response featuring decreased feed intake and impaired growth, inflammation with associated changes to its thermoregulation [7]. Its weight loss component is not only reflected by suppressed appetite, but also by metabolic/endocrine modification induced by inflammatory cytokines [8].

The high-LPS-rich outer membrane of Gram-negative bacteria serves as an essential protective barrier and plays a critical role in host–pathogen interactions and antibiotics susceptibility [9]. The structure of LPS consists of lipid A, core oligosaccharides, and O-antigens that determines membrane permeation and resistance patterns determines membrane permeation and resistance patterns[10]. It imparts innate resistance to the majority of antibiotics and antimicrobial peptides and thus, is a key contributor towards the increasing worldwide prevalence of antimicrobial resistance (AMR) [11].

Gram-negative organisms are especially worrisome, with resistance to even the most recent compounds described [12]. As a result, there is increasing interest in the discovery of new antimicrobial agents acting specifically against pathogenic bacteria with minimal impact on the commensal microbiota [13].

In this background, bacteriocins have gained prominence as potential candidates. Such peptides usually have a relatively narrow antibacterial spectrum, low cytotoxicity, and high potential for treating MDR bacteria [14].

Nisin is a class Ia lantibiotic produced by Lactococcus lactis and has been extensively studied for its broad-based antimicrobial efficacy and safe nature [15]. Nisin is approved by the U.S. Food and Drug Administration (FDA) as a natural food preservative and is widely recognized for its effectiveness against various Gram-positive pathogens. Although traditionally considered ineffective against gram-negative bacteria, accumulating evidence suggests that nisin may also exhibit activity against Gram-negative bacteria [16].

Mechanistic data suggest that nisin is able to interact with LPS and disturb the outer membrane, most likely in strains with truncated LPS cores then enabling lipid II access or channel formation [17]. Moreover, findings from solid-state nuclear magnetic resonance (NMR) analyses and leakage assays have demonstrated that LPS chemical structure plays a significant role in the sensitivity of bacteria to nisin detecting those with rough forms is stronger [18].

Apart from its antimicrobial properties, nisin has a high immunomodulatory and anti-inflammatory effect [19]. Nisin was observed to demonstrate a reduction in excessive cytokine production and the regulation of immune cell signaling pathways [20]. These properties may exemplify its ability to sustain physiological homeostasis within the stressed animals and to improve resilience against immunologically challenging pathological agents [21]. In poultry, the immunomodulatory role of nisin have been associated with improvements in feed intake and growth performance. Evidence suggests that nisin improves growth performance, relives inflammation-related anorexia, regulates heat production, and alleviates dissipation in broiler chickens challenged immunologically [22].

Previous studies have established that dietary nisin supplementation improves growth performance and feed intake in the early rearing phase of broiler

Abbreviations-Cont'd

PVN: paraventricular nucleus
LHA: lateral hypothalamic area
VMH: ventromedial hypothalamus
DMN: dorsomedial nucleus
AMR: antimicrobial resistance
MDR: multidrug-resistant
NMR: nuclear magnetic resonance
HPA: hypothalamic–pituitary–adrenal
BBB: blood–brain barrier
FCR: Feed Conversion Ratio
TNF-α: tumor necrosis factor-alpha
IL-1β:interleukin-1 beta
IL: interleukin
NPY: neuropeptide Y
AgRP: agouti-related peptide
POMC: pro-opiomelanocortin
CART: cocaine- and amphetamine-regulated transcript
FDA: Food and Drug Administration

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

chickens [23]. However, despite substantial evidence supporting its antimicrobial and immunological activities, the central mechanisms through which nisin influences feeding behavior, especially under conditions of LPS-induced inflammatory stress, are still inadequately known. Moreover, the effects of nisin on body temperature regulation, energy balance, and serum biochemical parameters have not been clearly elucidated.

Consequently, the present study was conducted to investigate the central effects of nisin on feeding behavior, water intake, body temperature, and serum parameters in lipopolysaccharide-challenged broiler chickens. More specifically, we examined intracerebroventricularly infused nisin, which may affect feed intake, rescue the LPS-dependent decrease in feed consumption, alter temperature and serum parameters, and lower the negative LPS effect. This research was also conducted to further elucidate the potential for nisin to serve as a functional feed additive for broiler chickens.

Results

Base on the results, ICV administration of an effective dose of nisin significantly increased cumulative feed intake compared with the control group at 90, 120, and 180 minutes post injection ($p \leq 0.05$). In contrast, ICV administration of an effective dose of LPS significantly decreased cumulative feed intake at 30, 60, 90, 120, and 180 minutes post injection compared with the control group ($p \leq 0.05$). Co-administration of nisin and LPS, significantly improved the LPS-induced reduction in feed intake, especially at 90, 120, and 180 minutes post injection ($p \leq 0.05$) (Figure 1).

Effects of ICV administration of nisin significantly reduced water intake at 30, 60, 90, 120, and 180 minutes post injection ($p \leq 0.05$); ICV administration of LPS also significantly reduced water intake at 30, 60, 90, 120, and 180 minutes post injection ($p \leq 0.05$). Co-administration of nisin and LPS, exhibited a greater reduction in water intake compared to the other groups ($p \leq 0.05$) (Figure 2).

As concerns body temperatures, no significant differences were observed between the nisin-treated and control groups ($p > 0.05$). In contrast, following LPS injection, body temperatures initially decreased and subsequently increased above the normal values. Co-administration of nisin with LPS significantly attenuated the LPS-induced hyperthermia ($p \leq 0.05$) (table 1).

The effects of the treatments on serum parameters revealed that there were no significant differences in the nisin-injected groups compared with the control group ($p > 0.05$). However, in the LPS-treated groups, ALB concentrations were significantly lower than those of the control group ($p \leq 0.05$). A similar reduction in ALB levels was also observed in the nisin and LPS co-treated groups ($p \leq 0.05$), illustrating the inability of nisin to inhibit the reduction of ALB caused by LPS. Conversely, the value of both AST and ALT were significantly higher in the LPS-treated groups compared with the control groups ($p \leq 0.05$), whereas in the nisin and LPS co-treated groups, nisin was able to reduce the impact of LPS on the mentioned serum parameters ($p \leq 0.05$) (table 2).

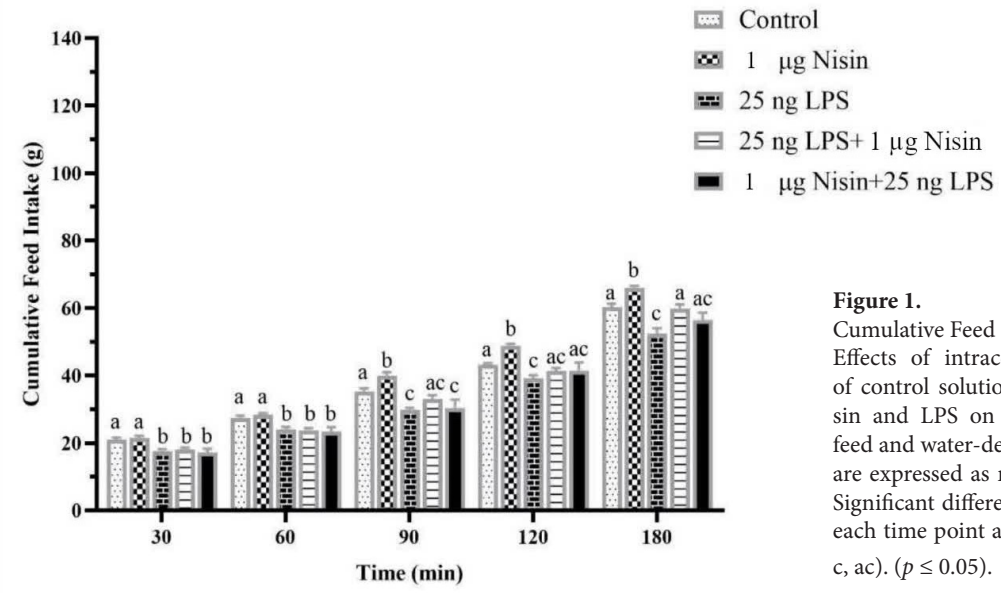

Figure 1.
Cumulative Feed Intake
Effects of intracerebroventricular injection of control solution and effective dose of nisin and LPS on cumulative feed intake in feed and water-deprived broilers (N=6). Data are expressed as mean ± standard deviation. Significant differences between treatments at each time point are indicated by letters (a, b, c, ac). ($p \leq 0.05$).

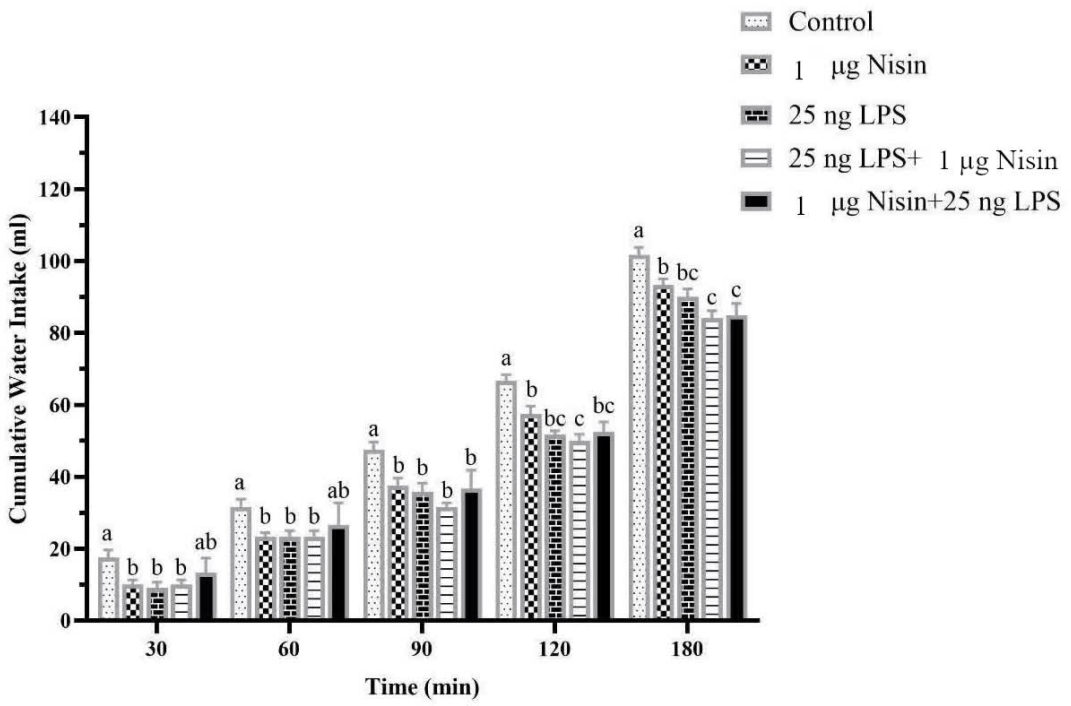

Figure 2.
Cumulative Water Intake
Effects of intracerebroventricular injection of control solution and effective dose of nisin and LPS on cumulative water intake in feed and water-deprived broilers (N=6). Data are expressed as mean ± standard deviation. Significant differences between treatments at each time point are indicated by letters (a, b, ab, bc, c). ($p \leq 0.05$).

Table 1.
Effects of nisin and LPS on body Temperature in the experiment.

Groups	10 min before inj.	1 h after inj.	3.5 h after inj.	5 h after inj.	8 h after inj.
1	40.76 ± 0.07^{a}	40.83 ± 0.04^{a}	40.78 ± 0.10^{a}	40.83 ± 0.06^{a}	40.83 ± 0.09^{a}
2	40.75 ± 0.05^{a}	40.76 ± 0.05^{a}	40.75 ± 0.08^{a}	40.83 ± 0.09^{a}	40.73 ± 0.06^{a}
3	40.86 ± 0.14^{a}	40.05 ± 0.07^{b}	40.88 ± 0.08^{a}	41.25 ± 0.09^{b}	42.11 ± 0.08^{b}
4	40.85 ± 0.11^{a}	39.86 ± 0.10^{b}	40.83 ± 0.07^{a}	40.95 ± 0.08^{ab}	41.16 ± 0.03^{c}
5	40.78 ± 0.06^{a}	39.76 ± 0.09^{b}	40.68 ± 0.11^{a}	40.85 ± 0.09^{a}	40.93 ± 0.06^{ac}

Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments at each time ($p \leq 0.05$).

Discussion

Nisin is a polypeptide bacteriocin produced by Lactococcus species, capable of improving broiler growth performance while exhibiting antioxidant and anti-inflammatory properties, which has led to growing interest in its application [24]. LPS, a component of Gram-negative bacteria, is commonly used to induce systemic inflammation in experimental animals. Previous studies have shown that LPS challenge significantly reduces weight gain, feed intake, and feed conversion ratio [25]. The present study is investigating the effects of ICV administration of nisin on feed intake in LPS-challenged broilers. Compared with the extensive research on nisin activity against Gram-positive bacteria, relatively few studies have addressed its effects on Gram-negative bacteria and their associated inflammation.

The results demonstrated that ICV administration of nisin increased feed intake at 90, 120, and 180 minutes post-injection. These finding are consistent with previous studies demonstrating that dietary supplementation with nisin enhances growth performance

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

Table 2.
Effects of nisin on some serum biochemical parameters of LPS-stimulated broilers

Groups	Cre	GLU	TG	Urea	BUN	ALP	ALB	AST	ALT
Control	0.23 ± 0.02	271.00 ± 1.71	77.00 ± 2.44	5.83 ± 0.16	2.67 ± 0.21	2763.00 ± 225.99	1.78 ± 0.04^{a}	274.33 ± 7.46^{a}	8.33 ± 0.42^{a}
Nisin	0.28 ± 0.01	266.33 ± 4.45	75.00 ± 2.17	5.17 ± 0.30	2.33 ± 0.21	2815.00 ± 175.95	1.78 ± 0.04^{a}	262.83 ± 4.50^{a}	8.17 ± 0.60^{a}
LPS	0.25 ± 0.02	255.67 ± 3.94	76.33 ± 3.45	5.00 ± 0.36	2.33 ± 0.21	2793.67 ± 241.46	1.35 ± 0.04^{b}	296.33 ± 2.40^{b}	10.17 ± 0.30^{b}
LPS + Nisin	0.27 ± 0.02	265.67 ± 7.81	76.83 ± 3.57	5.00 ± 0.25	2.33 ± 0.21	2712.33 ± 231.66	1.40 ± 0.03^{b}	278.83 ± 3.19^{ab}	9.83 ± 0.30^{ab}
Nisin + LPS	0.25 ± 0.02	264.33 ± 8.07	76.17 ± 3.68	4.83 ± 0.30	2.50 ± 0.22	2705.83 ± 231.24	1.42 ± 0.06^{b}	276.50 ± 2.26^{a}	9.50 ± 0.22^{ab}

Data are expressed as mean ± SEM. Abbreviations: Cre: Creatinine, GLU: Glucose, TG: Triglycerides, Urea: Urea, BUN: Blood Urea Nitrogen, ALB: Albumin, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase. Different letters (a, b and c) indicate significant differences between each treatment in the respective factors ($p \leq 0.05$).

and feed intake in broiler chickens, including Ross 308 strains [26]. In group 5 of the experiment, nisin was administered first, followed by LPS injection, to evaluate the potential preventive effects of nisin.

In the present study, it can be observed that the initial administration of nisin was effective in reducing the adverse effects of LPS, as it partially reduced the effects of LPS on food and water intake, temperature, and even some serum parameters. This can be related to earlier studies showing that nisin suppresses the Production of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [27], while promoting the production of regulatory mediators such as IL-10 and TGF-β [28].

In another study, nisin supplementation increased intestinal villus height, which may further contribute to improved broiler growth performance [23].

In another study, nisin supplementation increased intestinal villus height, which may further contribute to improved broiler growth performance [24]. In the present study, ICV administration of LPS reduced feed intake, consistent with previous studies .The faster and stronger effects observed via the ICV route may be explained by bypassing the blood–brain barrier (BBB) and directly influencing hypothalamic nuclei and neuropeptides related to hunger and satiety, such as the arcuate nucleus (ARC) and paraventricular nucleus (PVN), through modulation of neuropeptides including NPY, AgRP, POMC, and CART [29].

LPS has a damaging effect on intestines and their barrier, which could be a reason for lowered growth rates in LPS-inoculated broilers. Previous studies have shown that LPS affects the gene and protein structure of tight junctions in the intestines, thereby compromising nutrient absorption and water-electrolytes secretion [30]. The pro-inflammatory cytokine TNF-α, produced as a result of exposure to LPS, further contribute to the deterioration of tight junction proteins [31].

In broiler chickens, LPS-triggered anorexia has been mediated by decreased expression of orexigenic neuropeptides, such as Neuropeptide Y (NPY) and Agouti-Related Peptide (AgRP) in the hypothalamus, as well as activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis, along with changes in nutrient partitioning from growth to immune processes, such as antibody production and inflammatory cytokines [32].

In our experiment, the co-administration of LPS and nisin suggested that nisin has the potential to counteract the adverse effects of LPS on feed intake. The previous study has already validated that nisin has anti-inflammatory activity and nisin supplementation can suppress the Production of TNF-α and IL-6, while simultaneously promote the Production of IL-2, indi-

cating the stimulation of innate immunity [27].

In this study, besides the effect of nisin on cumulative feed intake, water intake was also measured. By comparison with energy balance, water balance or the regulation of body fluids for homeostasis is more complex as it includes a variety of biological functions such as the cellular and molecular regulation of water intake and excretion [33]. Various organs can also be involved in osmoregulation and fluid regulation. In birds, these organs include the kidney, intestine, and salt gland [34].

In the peresent study, ICV injections of nisin were associated with a significant reduction in water intake. The relationship between feed and water intake in the LPS-injected group was linear; however, this pattern was not observed in the nisin-treated group. The inverse association between feed and water intake, which is less common in poultry, may be influenced by several factors, including breed, environmental temperature, sex, growth rate, diet composition, and physicochemical properties of feed ingredients [35]. Additionally, the stimulation of specific nuclei in the central nervous system could potentially promote appetite and, at the same time, limit water consumption [36].

The challenge, illustrated by the conflicting effects on feed and water intake, indicates that nisin potentially impacts water intake in a unique way via the central nervous system. The impact of nisin on water intake in poultry has not been documented earlier, and in a study on nisin intake in mice, the findings illustrated an increase in water intake [37].

The paraventricular nucleus, supraoptic nucleus, and medial-basal hypothalamus are involved in the production and stimulation of neuropeptide Y, which occurs during osmotic stress and dehydration and these nuclei likely contribute to water regulation in chicks [38]. Because the paraventricular nucleus receives signals related to blood volume status, it may involve in the inhibition of NPY production in nucleus, and therefore it can play a role in stimulating drinking behavior [39].

Although both nisin and LPS caused reduced water intake, and co-administered nisin and LPS caused further reduced water intake as opposed to controls, the processes and physiological effects of nisin and LPS might not be the same.

Also, intestinal health is significant for water and electrolyte uptake in poultry and is very important for water balance [40]. Intestinal disorders or inflammation can result in impaired water uptake, causing diarrhea, thereby increasing water consumption and enteric infections also facilitate water excretion in the feces, apart from causing disturbances in electrolyte balance[24]. Nisin, with the capacity to reduce inflammation of the epithelial lining, causing a modification in the intestinal microbiota, can be very effective in the uptake of sodium, thus potentially reducing water consumption[27].

Overhydration in broilers may lead to increased moisture and saturated bedding that makes the broiler flocks prone to diseases such as ascites and contact dermatitis [41]. Adequate regulation of water consumption is also important to lower the chances of developing physiological ailments such as tibial dyschondroplasia, ascites, cardiovascular diseases, and

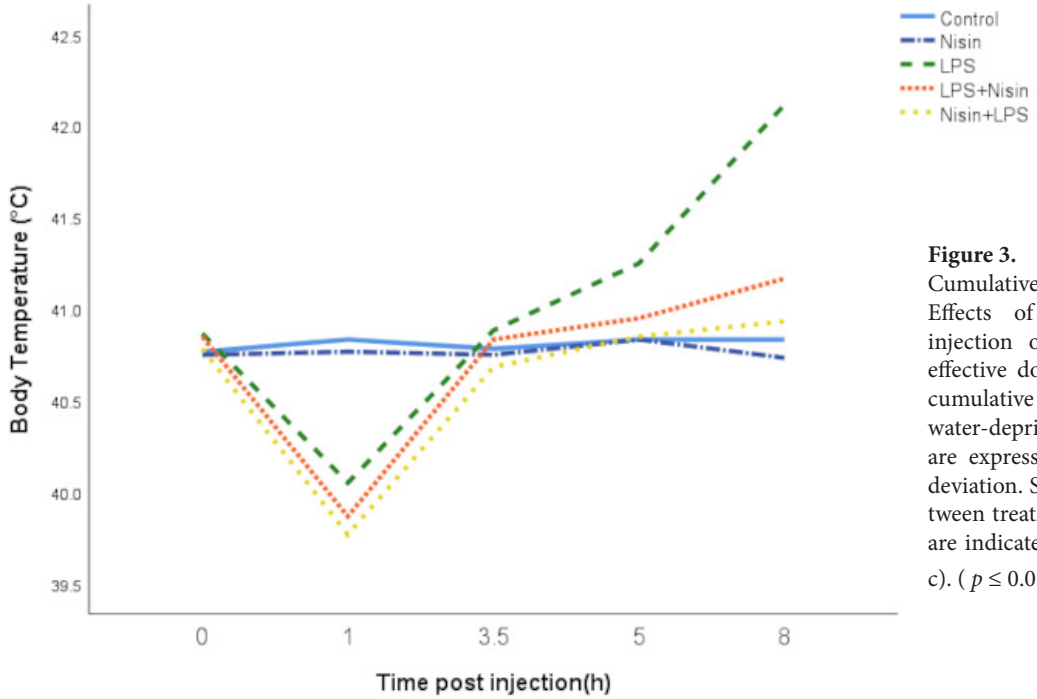

Figure 3.
Cumulative Water Intake
Effects of intracerebroventricular injection of control solution and effective dose of nisin and LPS on cumulative water intake in feed and water-deprived broilers (N=6). Data are expressed as mean ± standard deviation. Significant differences between treatments at each time point are indicated by letters (a, b, ab, bc, c). ($p \leq 0.05$).

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

reproductive failure[42]. In our study, cloacal temperature measurements 10 minutes before the start of the experiment and 1, 3.5, 5, and 8 hours after injection confirmed the effects of LPS. Showing an initial drop followed by increased temperature in LPS-treated groups. Similar findings have been reported in earlier studies, where LPS IV-injection elevated cloacal temperature three hours post-administration [43]. Another study also observed increased body temperature and reduced appetite in LPS-challenged broilers and reported that strong LPS doses initially decreased then increased body temperature in chickens [44]. Notably, in the present study, nisin was able to somewhat reduce the adverse effects of LPS on body temperature among the intervention groups. Figure 3 shows the temperature changes in each experimental group during the experiment, and in Table 1, the groups are compared at different times, and the numbers are written as mean ± SEM.

The results of serum biochemical parameters, did not show significant differences between the nisin-treated and control group. In another study that investigated the effects of nisin on the blood serum of broiler chickens, no significant changes were observed in serum parameters except glucose [26]. The differences in the effects of the variables related to glucose may be attributed to different factors such as the duration of the experiment, the differences in the dose, and the experimental conditions. In another experiment conducted on rats, it was found that the consumption of nisin did not affect the liver enzymes AST and ALT, triglycerides, cholesterol, HDL, and LDL, which demonstrates that nisin is not harmful to the physiological health of the human body[45]. In a study on rabbits, nisin administered in drinking water did not lead to significant differences in total protein, triglycerides, cholesterol, glucose, calcium or ALT[46]. In the present study, the results of serum biochemical parameters in the LPS group showed an increase in the levels of ALT and AST enzymes. The activities of AST and ALT enzymes in serum are usually used as specific indicators of liver damage. The results of other studies showed that intraperitoneal injection of LPS increased the levels of ALT and AST [47]. Notably. in the present study, albumin levels decreased, and reduction in albumin concentration in the LPS group may also be attributed to liver injury.

The immune response in broiler chickens caused by LPS negatively affects their growth; this occurs mainly because of protein metabolism failure in the body. The results in the intervention groups show that nisin can significantly reduce the adverse effects of LPS on ALT and AST factors, but has no effect on the adverse effects of LPS on ALB factor.

Conclusions

This study shows that central injection of nisin can increase feed intake and reduce water consumption in broiler chickens, and reduces the negative effects of LPS on feed intake, body temperature, and some serum parameters. These findings provide new evidence for the potential role of bacteriocins, particularly nisin, in modulating feed and water intake and reducing the deleterious effects on body physiology in LPS-induced broiler chickens.

Materials and Methods

Ethical Considerations

The present study was conducted in accordance with ethical principles, national regulations, and standards for medical research in Iran.

Ethical approval ID: IR.UM.REC.1403.313, dated January 25, 2025.

All experiments were executed according to the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal ethics committee.

Animals

A total of 30 one-day-old male broiler chicks of the Ross 308 breed were obtained from Fariman Company (Mashhad, Iran). During the first five days post-hatch, birds were maintained under normal rearing conditions. Broilers were housed in a controlled environment with temperatures of 30 ± 2° C for the initial 48-hour period, followed by a gradual reduction of approximately 2.5° C per week. Relative humidity was maintained at 50 ± 2%.

The lighting cycle adhered to conventional management practices, consisting of 23 hours light and 1 hour darkness during the first week, and gradually adjusted to a 18 hours light and 6 hour darkness photoperiod by the end of the experiment period. The broilers had free access to clean water and a commercial broiler starter feed (Sāleh Co., Kashmar) throughout the experiment.

The diet was formulated with a concentration of 23% crude protein, 2% crude fiber, 1% calcium, 0.5% available phosphorus, 0.55% methionine, 1% lysine, 1% methionine + cystine, 0.18% and 2900 kcal/kg metabolizable energy.

After the 5-day acclimation time, chicks were randomly allocated to five experimental groups (n = 6 per group). Prior to intracerebroventricular (ICV) injections, birds were fasted for 3 hours to minimize variation in metabolic status, while water remained available ad libitum.

Experimental Compounds

Nisin (Product Code: N5764-1G) was purchased from Sigma-Aldrich, USA. This bioactive compound, which belongs to Lactococcus lactis, has been defined to consist of 2.5% purified nisin along with sodium chloride and denatured milk derived components. This bioactive compound, manufactured and sold by Sigma-Aldrich, has been registered and indexed using CAS number 1414-45-5, and it possesses an empirical formula composed of $C_{143}H_{230}N_{42}O_{37}$. Lipopolysaccharide (LPS) was obtained from E. coli O111:B4 (Life Biolab, Cat. No. LB22429, Lot No. 20L9147).

All solutions were prepared using sterile, pyrogen-free physiological saline. A stock solution with 1 mg/ml nisin was prepared

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

and subsequently diluted to obtain the desired experimental concentration (1 µg). A stock solution with 10 mg/ml LPS was prepared and subsequently diluted to obtain the desired experimental concentration.

Experimental Groups
Group 1: Control
Group 2: Nisin
Group 3: LPS
Group 4: LPS + Nisin
Group 5: Nisin + LPS

The time interval between the two injections of nisin and LPS in the fourth and fifth groups was 15 minutes.

Surgical Preparation

Stereotactic surgery was performed when the chicks were 18 days old and weighed approximately 750g. Food was withdrawn for one hour before surgery, while water w remained ad libitum. Anesthesia was induced via a face mask using isoflurane (5% isoflurane in 1 L/min oxygen) and maintained at 2–3% isoflurane (Piramal Critical Care Inc., Guayama, USA) in 1 L/min oxygen delivered through a tracheal tube. All broilers were confirmed to be clinically healthy following routine physical examination.

A 23-gauge thin-walled stainless steel guide cannula was stereotaxically implanted into the right lateral ventricle according to previously established coordinates: 6.7 mm anterior to bregma, 0.7 mm lateral to the midline, and 3.5-4 mm below the dura mater [48]. Finally, three stainless steel screws were inserted around the entry point of the cannula into the skull, and the cannula and the three screws were fixed in place with acrylic dental cement (Acropars, Tehran, Iran). In the control group, an orthodontic #014 wire was inserted into the guide cannula to maintain equivalent conditions.

To prevent postoperative infection, Linkospectin (Razak, Tehran, Iran) was applied topically to the incision site and administered intraperitoneally. Birds were allowed a minimum recovery period of five days, before the initiation of the subsequent injections.

Experimental Procedures

A single experiment was conducted to evaluate the effects of nisin on feed intake, water intake, body temperature, and serum biochemical parameters in broilers challenged with LPS-induced inflammation (n = 30). Each experiment consisted of one control group and four treatment groups. Body weight (BW) was consistent across all chickens (950 ± 50 g).

The injections were administered using a thin-walled stainless steel injection cannula of 30-gauge, which projected 1.0 mm beyond the tip of the guide cannula. The injection cannula was connected to a 10-µl Hamilton syringe with a 30-cm PE-20 polyethylene tube. The Lipopolysaccharide was given an infusion time of 60 seconds, and the control solution was infused into the lateral ventricle simultaneously. All tubes and syringes were stored in a 70% ethanol. Glassware was sterilized by an autoclave to maintain a Pyrogen-free environment. Sterilized physiological saline was used as a control solution.

Correct placement of the guide cannula within the right lateral ventricle was confirmed by the appearance of cerebrospinal fluid (CSF) and by ICV infusion of methylene blue, followed by methylene blue in the right place confirmation in frozen brain sections at the conclusion of each experiment. Only data from accurately performed injections were included in the statistical analyses.

Feed was withheld 3 hours prior to the start of the experiment. The chicken has been immediately returned into the cage after injection. The cumulative feed consumption (g) and water consumption (g) of the chicken were measured at 30 min, 60 min, 90 min, 120 min, and 180 min post-injection. At the time of experimentation, chicks were 23 days old.

Nisin and LPS were administered via the ICV route alone or in combination to assess the central nisin administration on LPS-induced inflammation. Chickens received 1 µg/kg of nisin dissolved in 10 µl saline. LPS was administered at a dose of 25 ng in 10 µl saline.

Because no prior studies have reported ICV administration of nisin in broilers, the selected dose was based on data from mammalian studies and preliminary pilot experiments conducted by the current research team. In contrast, the effective ICV dose of LPS in broilers (25 ng) has been previously established by some of the members of the present research team [49]. At the end of the experiments, blood samples were collected from the metatarsal vein.

Statistical Analysis

The cumulative amount of feed and water intake data was analyzed employing one-way repeated measures ANOVA in GraphPad Prism software, version 9.4.1 (GraphPad Software, USA). In order to determine statistical significance, the value of $p \leq 0.05$ was taken into consideration. After observing significant differences in treatment, the Tukey post-test comparison was conducted.

Variations that might exist between the body temperatures of the LPS-treated and control broilers and the serum biochemical values were estimated using the one-way ANOVA method. Statistical significance was accepted at $p \leq 0.05$.

Authors' Contributions

All authors contributed to the study conception and design. Vahid Hamedianasl performed material preparation, data collection, and investigation. Farshid Hamidi carried out project administration, supervision, methodology, conceptualization, review and editing of the manuscript. Hamidreza Kazerani carried out the formal analysis, software processing, validation, and visualization. Vahid Hamedianasl wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank you to Ferdowsi University of Mashhad, for their support.

Competing Interests

The authors declare that there is no conflict of interest.

Nisin, LPS, Feeding behavior, Temperature, Serum parameters, Broilers

Reference

1. Saneyasu T. Recent research on mechanisms of feeding regulation in chicks. The Journal of Poultry Science. 2024;61:2024012. Doi:10.2141/jpsa.2024012.

2. Volyanskaya AR, Akberdin IR, Kulyashov MA, Yevshin IS, Romanov MN, Shagimardanova EI, et al. A bird's-eye overview of molecular mechanisms regulating feed intake in chickens—with mammalian comparisons. Animal Nutrition. 2024;17:61–74. Doi:10.1016/j.aninu.2024.01.008.

3. Li Z, Liu X, Zhang P, Han R, Sun G, Jiang R, et al. Comparative transcriptome analysis of hypothalamus-regulated feed intake induced by exogenous visfatin in chicks. BMC Genomics. 2018;19(1):249. Doi:10.1186/s12864-018-4644-7.

4. Brugaletta G, Greene E, Tabler T, Orlowski S, Sirri F, Dridi S. Effect of cyclic heat stress on feeding-related hypothalamic neuropeptides of three broiler populations and their ancestor jungle fowl. Frontiers in Physiology. 2021;12:809341. Doi:10.3389/fphys.2021.809341.

5. Zmrhal V, Svoradova A, Venusova E, Slama P. The influence of heat stress on chicken immune system and mitigation of negative impacts by Baicalin and Baicalein. Animals. 2023;13(16):2564. Doi:10.3390/ani13162564.

6. Kikusato M, Toyomizu M. Mechanisms underlying the effects of heat stress on intestinal integrity, inflammation, and microbiota in chickens. The Journal of Poultry Science. 2023;60(2):2023021. Doi:10.2141/jpsa.2023021.

7. Al-Zghoul MB, Hundam S, Mayyas M, Gerrard DE, Dalloul RA. Impact of Thermal Manipulation of Broiler Eggs on Growth Performance, Splenic Inflammatory Cytokine Levels, and Heat Shock Protein Responses to Post-Hatch Lipopolysaccharide (LPS) Challenge. Animals. 2025;15(12):1736. Doi:10.3390/ani15121736.

8. Choi J, Lee J, Kim WK. Changes in gene and protein expression related to feed intake and thermoregulation in broilers challenged with different doses of mixed Eimeria spp. Poultry Science. 2025:105481. doi:10.1016/j.psj.2025.105481

9. May KL, Akiyama T, Parker BG, Kim M, Grabowicz M. LPS O-antigen polysaccharide length impacts outer membrane permeability of enteric gram-negative bacteria. mBio. 2025:e02518–25. Doi:10.1128/mbio.02518-25.

10. Chen H, Siroy A, Morales V, Quentin Y, Balor S, Abuta'a YA, et al. Structural basis of lipopolysaccharide assembly by the outer membrane translocon holo-complex. Nature Communications. 2025;16(1):10404. Doi:10.1038/s41467-025-65370-2.

11. Hickson SM, Ledger EL, Wells TJ. Emerging antimicrobial therapies for Gram-negative infections in human clinical use. npj Antimicrobials and Resistance. 2025;3(1):16. Doi:10.1038/s44259-025-00087-2.

12. Jia R, Xiao C-x, Zhang Y-h, Hu L-y, Jun-Jun Y, Zuo R, et al. Microbiota in drug resistance. Drug Resistance Updates. 2025:101311. Doi:10.1016/j.drup.2025.101311.

13. Aunpad R, Thitirungreangchai T. Advancing antimicrobial peptides: Overcoming challenges in the era of bacterial resistance. Biochimie. 2025;238(B):1-19. Doi:10.1016/j.biochi.2025.07.019.

14. Bisht V, Das B, Hussain A, Kumar V, Navani NK. Understanding of probiotic origin antimicrobial peptides: a sustainable approach ensuring food safety. npj Science of Food. 2024;8(1):67. Doi:10.1038/s41538-024-00304-8.

15. Shin JM, Gwak JW, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Biomedical applications of nisin. Journal of Applied Microbiology. 2016;120(6):1449–65. Doi:10.1111/jam.13033.

16. Wang X, Kang W, Li J, Deng Z, Gao J. Nisin: harnessing nature's preservative for the future of food safety and beyond. Critical Reviews in Food Science and Nutrition. 2025;65(34):9045-70. Doi:10.1080/10408398.2025.2517822.

17. Münch D, Sahl H-G. Structural variations of the cell wall precursor lipid II in Gram-positive bacteria—Impact on binding and efficacy of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2015;1848(11):3062–71. Doi:10.1016/j.bbamem.2015.04.014.

18. Vukomanović M, Žunič V, Kunej Š, Jančar B, Jeverica S, Podlipec R, et al. Nano-engineering the antimicrobial spectrum of lantibiotics: activity of nisin against gram negative bacteria. Sci Rep. 2017;7(1):4324. Doi:10.1038/s41598-017-04670-0.

19. Huang F, Teng K, Liu Y, Wang T, Xia T, Yun F, et al. Nisin Z attenuates lipopolysaccharide-induced mastitis by inhibiting the ERK1/2 and p38 mitogen-activated protein kinase signaling pathways. Journal of Dairy Science. 2022;105(4):3530–43. Doi:10.3168/jds.2021-21356.

20. Małaczewska J, Kaczorek-Łukowska E. Nisin—A lantibiotic with immunomodulatory properties: A review. Peptides. 2021;137:170479. Doi:10.1016/j.peptides.2020.170479.

21. Sun Y, Yan T, Gong G, Li Y, Zhang J, Wu B, et al. Antidepressant-like effects of Schisandrin on lipopolysaccharide-induced mice: Gut microbiota, short chain fatty acid and TLR4/NF-κB signaling pathway. International Immunopharmacology. 2020;89:107029. Doi:10.1016/j.intimp.2020.107029.

22. Li Y, Wang Y, Yan S, Li Y, Gao X, Yu J, et al. Nisin A elevates adenosine to achieve anti-inflammatory activity. Food & Function. 2024;15(20):10490–503. Doi:10.1039/d4fo01834f.

23. Józefiak D, Kierończyk B, Juśkiewicz J, Zduńczyk Z, Rawski M, Długosz J, et al. Dietary nisin modulates the gastrointestinal microbial ecology and enhances growth performance of the broiler chickens. PloS one. 2013;8(12):e85347. Doi:10.1371/journal.pone.0085347.

24. Yuan H, Bai G, Lin Y, Yu X, Yang Q, Dou R, et al. Effects of dietary Nisin on growth performance, immune function, and gut health of broilers challenged by Clostridium perfringens. Journal of Animal Science. 2024 Jan 1;102:skae017. Doi:

10.1093/jas/skae017.

25. Dias KM, Oliveira CH, Calderano AA, Rostagno HS, Gomes KM, O'Connor KE, et al. Dietary hydroxytyrosol supplementation on growth performance, gut morphometry, and oxidative and inflammatory status in lps-challenged broilers. Animals. 2024;14(6):871. Doi:10.3390/ani14060871.

26. Kierończyk B, Sassek M, Pruszyńska-Oszmałek E, Kołodziejski P, Rawski M, Świątkiewicz S, et al. The physiological response of broiler chickens to the dietary supplementation of the bacteriocin nisin and ionophore coccidiostats. Poultry Science. 2017;96(11):4026–37. Doi:10.3382/ps/pex234.

27. Uehara A, Maekawa M, Nakagawa K. The mechanism of action of nisin in promoting poultry through tight junction integrity and dendritic cell anti-inflammatory properties. Archives of Microbiology. 2025;207(7):165. Doi:10.1007/s00203-025-04364-6.

28. Obianwuna UE, Agbai Kalu N, Wang J, Zhang H, Qi G, Qiu K, et al. Recent trends on mitigative effect of probiotics on oxidative-stress-induced gut dysfunction in broilers under necrotic enteritis challenge: a review. Antioxidants. 2023;12(4):911. Doi:10.3390/antiox12040911.

29. Tachibana T, Tsutsui K. Neuropeptide control of feeding behavior in birds and its difference with mammals. Frontiers in Neuroscience. 2016;10:485. Doi:10.3389/fnins.2016.00485.

30. Tan H, Zhen W, Bai D, Liu K, He X, Ito K, et al. Effects of dietary chlorogenic acid on intestinal barrier function and the inflammatory response in broilers during lipopolysaccharide-induced immune stress. Poultry Science. 2023;102(5):102623. Doi:10.1016/j.psj.2023.102623.

31. Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal barrier dysfunction, LPS translocation, and disease development. Journal of the Endocrine Society. 2020;4(2):bvz039. Doi:10.1210/jendso/bvz039.

32. Liu K, Zhen W, Bai D, Tan H, He X, Li Y, et al. Lipopolysaccharide-induced immune stress negatively regulates broiler chicken growth via the COX-2-PGE2-EP4 signaling pathway. Frontiers in Immunology. 2023;14:1193798. Doi:10.3389/fimmu.2023.1193798.

33. Hughes M. Regulation of salt gland, gut and kidney interactions. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2003;136(3):507–24. Doi:10.1016/j.cbpb.2003.09.005.

34. McWhorter TJ, Caviedes-Vidal E, Karasov WH. The integration of digestion and osmoregulation in the avian gut. Biological Reviews. 2009;84(4):533–65. Doi:10.1111/j.1469-185x.2009.00086.x.

35. Carré B, Lessire M, Juin H. Prediction of metabolisable energy value of broiler diets and water excretion from dietary chemical analyses. Animal. 2013;7(8):1246–58. Doi:10.1017/s1751731113000359.

36. Menezes ACB, Valadares Filho SC, Benedeti PD, Zanetti D, Paulino MF, Silva FF, et al. Feeding behavior, water intake, and energy and protein requirements of young Nellore bulls with different residual feed intakes. Journal of Animal Science. 2020;98(9):skaa279. Doi:10.1093/jas/skaa279.

37. Hagiwara A, Imai N, Nakashima H, Toda Y, Kawabe M, Furukawa F, et al. A 90-day oral toxicity study of nisin A, an anti-microbial peptide derived from Lactococcus lactis subsp. lactis, in F344 rats. Food and Chemical Toxicology. 2010;48(8-9):2421–8. Doi:10.1016/j.fct.2010.06.002.

38. Bohler MW, Chowdhury VS, Cline MA, Gilbert ER. Heat stress responses in birds: A review of the neural components. Biology. 2021;10(11):1095. Doi:10.3390/biology10111095.

39. Nagarajan G, Kang SW, Kuenzel WJ. Functional evidence that the nucleus of the hippocampal commissure shows an earlier activation from a stressor than the paraventricular nucleus: Implication of an additional structural component of the avian hypothalamo-pituitary-adrenal axis. Neuroscience letters. 2017;642:14–9. Doi:10.1016/j.neulet.2017.01.064.

40. Kierończyk B, Rawski M, Mikołajczak Z, Świątkiewicz S, Józefiak D. Nisin as a novel feed additive: the effects on gut microbial modulation and activity, histological parameters, and growth performance of broiler chickens. Animals. 2020;10(1):101. Doi:10.3390/ani10010101.

41. Manning L, Chadd S, Baines R. Key health and welfare indicators for broiler production. World's Poultry Science Journal. 2007;63(1):46–62. Doi:10.1017/S0043933907001262.

42. D'Eath RB, Tolkamp BJ, Kyriazakis I, Lawrence AB. 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. Animal Behaviour. 2009;77(2):275–88. Doi:10.1016/j.anbehav.2008.10.028.

43. Xie H, Rath N, Huff G, Huff W, Balog J. Effects of Salmonella typhimurium lipopolysaccharide on broiler chickens. Poultry Science. 2000;79(1):33–40. Doi:10.1093/ps/79.1.33.

44. De Boever S, Beyaert R, Vandemaele F, Baert K, Duchateau L, Goddeeris B, et al. The influence of age and repeated lipopolysaccharide administration on body temperature and the concentration of interleukin-6 and IgM antibodies against lipopolysaccharide in broiler chickens. Avian Pathology. 2008;37(1):39–44. Doi:10.1080/03079450701784875.

45. Al-Emarah M, Kazerani HR, Taghizad F, Dehghani H, Elahi M. Anti-obesity effect of the bacterial product nisin in an NIH Swiss mouse model. Lipids in Health and Disease. 2023;22(1):23. Doi:10.1186/s12944-023-01788-1.

46. Lauková A, Chrastinová Ľ, Plachá I, Kandričáková A, Szabóová R, Strompfová V, et al. Beneficial effect of lantibiotic nisin in rabbit husbandry. Probiotics and Antimicrobial Proteins. 2014;6(1):41–6. Doi:10.1007/s12602-014-9156-4.

47. Yu Z, Guo F, Zhang Z, Luo X, Tian J, Li H. Protective Effects of Glycyrrhizin on LPS and Amoxicillin/Potassium Clavula-

nate-Induced Liver Injury in Chicken. Pakistan Veterinary Journal. 2017;37(1): 13-18..

48. Zendehdel M, Hamidi F, Hassanpour S. T. The effect of histaminergic system on nociceptin/orphanin FQ induced food intake in chicken. International Journal of Peptide Research and Therapeutics. 2015;21(2):179–186. Doi:10.1007/s10989-014-9445-5.

49. Rahdari A, Hamidi F. The effect of intraperitoneal injection of Glycyrrhizin on central regulation of food intake in broilers injected with LPS. British Poultry Science. 2025;66(1):124–30. Doi:10.1080/00071668.2024.2396451.

How to cite this article
Hamedianasl V, Hamidi F, Kazerani H. Effects of intracerebroventricular injection of nisin on feeding behavior, body temperature, and serum parameters in LPS-inflamed broiler chickens. Iran J Vet Sci Technol. 2026; 18(3): 30-40.
DOI: https://doi.org/10.22067/ijvst.2026.97643.1639
URL:https://ijvst.um.ac.ir/article_48292.html